

THAT WHICH IS CLAIMED:

1. A method of determining an amount of at least one molecular specie comprising a sample, each molecular specie being indicated by a dye, from an image of the sample captured as image data by a color image acquisition device in a video-microscopy system, said method comprising:

determining an optical density of the sample from the image data in each of a red, green, and blue channel at a pixel in the image so as to form a corresponding optical density matrix for the pixel; and

multiplying the optical density matrix by an inverse of a relative absorption coefficient matrix so as to form a resultant matrix for the pixel, the relative absorption coefficient matrix comprising a relative absorption coefficient for each dye, independently of the sample, in each of the red, green, and blue channels, the resultant matrix comprising the amount of each molecular specie, as indicated by the respective dye, for the pixel.

2. A method according to Claim 1 further comprising determining the relative absorption coefficient for each dye, independently of the sample, in each of the red, green, and blue channels so as to form the corresponding relative absorption coefficient matrix.

3. A method according to Claim 2 wherein determining the relative absorption coefficient further comprises determining an initial intensity of a light emitted by a light source in each of the red, green, and blue channels.

4. A method according to Claim 3 wherein determining the relative absorption coefficient further comprises illuminating each dye with the light source, independently of the sample, and determining a transmitted intensity of the light transmitted therethrough in each of the red, green, and blue channels.

5. A method according to Claim 4 wherein determining the relative absorption coefficient further comprises comparing the initial intensity of the light to the transmitted intensity of light so as to determine an optical density for each dye in each of the red, green, and blue channels.

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6. A method according to Claim 4 wherein determining the relative absorption coefficient further comprises determining an optical density for each dye by determining a natural logarithm of a ratio of the initial intensity of the light to the transmitted intensity of the light in each of the red, green, and blue channels.

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7. A method according to Claim 6 wherein determining the relative absorption coefficient further comprises normalizing the optical density in each of the red, green, and blue channels, with respect to the channel having the highest optical density, for each dye.

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8. A method according to Claim 3 wherein determining an optical density of the sample further comprises illuminating the sample with the light source and determining a transmitted intensity of the light transmitted therethrough in each of the red, green, and blue channels.

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9. A method according to Claim 8 wherein determining an optical density of the sample further comprises comparing the initial intensity of the light to the transmitted intensity of the light so as to determine an optical density for the sample in each of the red, green, and blue channels.

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10. A method according to Claim 8 wherein determining an optical density of the sample further comprises determining a natural logarithm of the ratio of the initial intensity of the light to the transmitted intensity of the light through the sample in each of the red, green, and blue channels.

11. A method according to Claim 1 further comprising capturing an image of the sample as image data in each of the red, green, and blue channels of at least one of an RGB camera and an RGB-configured scanner.

5 12. A method according to Claim 1 further comprising illuminating the sample under Koehler illumination conditions.

13. A method according to Claim 1 further comprising correcting chromatic aberration in the video-microscopy system.

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14. A method of determining the amount of each of a plurality of molecular species comprising a sample, each molecular specie being indicated by a dye, at least one dye comprising a marker dye and another dye comprising a counterstain, from an image of the sample captured as image data by a color camera in a video-microscopy system, said method comprising:

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determining an optical density of the sample from the image data in each of a red, green, and blue channel at a pixel in the image so as to form a corresponding optical density matrix for the pixel; and

multiplying the optical density matrix by an inverse of a relative absorption coefficient matrix so as to form a resultant matrix for the pixel, the relative absorption coefficient matrix comprising a relative absorption coefficient for each dye, independently of the sample, in each of the red, green, and blue channels, the resultant matrix comprising the amount of each molecular specie, as indicated by the respective dye, for the pixel.

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15. A method according to Claim 14 further comprising determining the relative absorption coefficient for at least the marker dye and the counterstain, independently of the sample, in each of the red, green, and blue channels so as to form the corresponding relative absorption coefficient matrix.

16. A method according to Claim 15 wherein determining the relative absorption coefficient further comprises determining an initial intensity of a light emitted by a light source in each of the red, green, and blue channels.

5 17. A method according to Claim 16 wherein determining the relative absorption coefficient further comprises illuminating each dye with the light source, independently of the sample, and measuring a transmitted intensity of the light transmitted therethrough in each of the red, green, and blue channels.

10 18. A method according to Claim 17 wherein determining the relative absorption coefficient further comprises comparing the initial intensity of the light to the transmitted intensity of light so as to determine an optical density for each dye in each of the red, green, and blue channels.

15 19. A method according to Claim 17 wherein determining the relative absorption coefficient further comprises determining the optical density for each dye by determining a natural logarithm of a ratio of the initial intensity of the light to the transmitted intensity of the light in each of the red, green, and blue channels.

20 20. A method according to Claim 19 wherein determining the relative absorption coefficient further comprises normalizing the optical density in each of the red, green, and blue channels, with respect to the channel having the highest optical density, for each dye.

25 21. A method according to Claim 16 wherein measuring an optical density of the sample further comprises illuminating the sample with the light source and measuring a transmitted intensity of the light transmitted therethrough in each of the red, green, and blue channels.

30 22. A method according to Claim 21 wherein measuring an optical density of the sample further comprises comparing the initial intensity of the light to the transmitted

intensity of the light so as to determine an optical density for the sample in each of the red, green, and blue channels.

23. A method according to Claim 21 wherein measuring an optical density of the sample further comprises calculating a natural logarithm of the ratio of the initial intensity of the light to the transmitted intensity of the light through the sample in each of the red, green, and blue channels.

24. A method according to Claim 14 further comprising illuminating the sample under Koehler illumination conditions.

25. A method according to Claim 14 further comprising correcting chromatic aberration in the video-microscopy system.

26. A video-microscopy system for determining an amount of at least one molecular specie comprising a sample, each molecular specie being indicated by a dye, from an image of the sample, said system comprising:

a color image acquisition device configured so as to be capable of capturing a magnified digital image of the sample as image data;

a computer device operably engaged with the image acquisition device and comprising:

a processing portion configured to determine an optical density of the sample from the image data in each of a red, green, and blue channel at a pixel in the image so as to form a corresponding optical density matrix for the pixel; and

a processing portion configured to multiply the optical density matrix by an inverse of a relative absorption coefficient matrix so as to form a resultant matrix for the pixel, the relative absorption coefficient matrix comprising a relative absorption coefficient for each dye, independently of the sample, in each of the red, green, and blue

channels, the resultant matrix comprising the amount of each molecular specie, as indicated by the respective dye, for the pixel.

27. A system according to Claim 26 wherein the image acquisition device
5 comprises at least one of a scanner and a microscope operably engaged with a color
camera.

28. A system according to Claim 26 further comprising a light source directed
toward the image acquisition device and configured to emit a light having an initial
10 intensity in each of the red, green, and blue channels.

29. A system according to Claim 28 wherein the light source is configured to illuminate the sample and the processing portion for determining optical density is further configured to direct a measurement of a transmitted intensity of the light transmitted through the sample in each of the red, green, and blue channels.

30. A system according to Claim 29 wherein the processing portion for determining optical density is further configured to compare the initial intensity of the light to the transmitted intensity of the light so as to determine an optical density for the sample in each of the red, green, and blue channels.

31. A system according to Claim 29 wherein the processing portion for determining optical density is further configured to calculate a natural logarithm of a ratio of the initial intensity of the light to the transmitted intensity of the light so as to
25 determine an optical density for the sample in each of the red, green, and blue channels.

32. A system according to Claim 28 wherein the computer device further comprises a processing portion configured to direct the light source to illuminate each dye, independently of the sample, and to direct a measurement of a transmitted intensity of the light transmitted therethrough in each of the red, green, and blue channels.

33. A system according to Claim 32 wherein the computer device further comprises a processing portion configured to compare the initial intensity of the light to the transmitted intensity of the light so as to determine an optical density for each dye in each of the red, green, and blue channels.

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34. A system according to Claim 32 wherein the computer device further comprises a processing portion configured to determine the optical density for each dye by determining a natural logarithm of a ratio of the initial intensity of the light to the transmitted intensity of the light in each of the red, green, and blue channels.

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35. A system according to Claim 34 wherein the computer device further comprises a processing portion configured to normalize the optical density in each of the red, green, and blue channels, with respect to the channel having the highest optical density, for each dye so as to determine the respective relative absorption coefficient in each of the red, green, and blue channels.

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36. A system according to Claim 26 wherein the computer device further comprises a storage media configured to store the relative absorption coefficient in each of the red, green, and blue channels for a plurality of dyes, and wherein the dyes indicating the molecular species in the sample are selected from the plurality of dyes.

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37. A system according to Claim 36 wherein the computer device further comprises a processing portion configured to retrieve the respective relative absorption coefficients for each dye from the storage media, and to form the corresponding relative absorption coefficient matrix, in accordance with the dyes indicating the molecular species in the sample which are selected from the plurality of dyes.

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38. A system according to Claim 26 wherein the computer device further comprises a processing portion configured to invert the relative absorption coefficient matrix.

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39. A system according to Claim 28 wherein the light source is further configured to provide Koehler illumination conditions.

40. A system according to Claim 26 wherein the image acquisition device is further configured to correct chromatic aberration.

41. A computer software program product capable of determining an amount of at least one molecular specie comprising a sample, each molecular specie being indicated by a dye, from a digital image of the sample captured as image data by a color image acquisition device in a video-microscopy system, said computer software program product being executable on a computer device and comprising:

an executable portion capable of determining an optical density of the sample from the image data in each of a red, green, and blue channel at a pixel in the digital image so as to form a corresponding optical density matrix for the pixel; and

an executable portion capable of multiplying the optical density matrix by an inverse of a relative absorption coefficient matrix so as to form a resultant matrix for the pixel, the relative absorption coefficient matrix comprising a relative absorption coefficient for each dye, independently of the sample, in each of the red, green, and blue channels, the resultant matrix comprising the amount of each molecular specie, as indicated by the respective dye, for the pixel.

42. A computer software program product according to Claim 41 further comprising an executable portion capable of directing a light source to illuminate the sample with a light having an initial intensity in each of the red, green, and blue channels.

43. A computer software program product according to Claim 42 further comprising an executable portion capable of directing a measurement of a transmitted intensity of the light transmitted through the sample in each of the red, green, and blue channels.

44. A computer software program product according to Claim 43 wherein the executable portion for determining an optical density is further capable of comparing the initial intensity of the light to the transmitted intensity of the light so as to determine an optical density for the sample in each of the red, green, and blue channels.

45. A computer software program product according to Claim 43 wherein the executable portion for determining an optical density is further capable of calculating a natural logarithm of a ratio of the initial intensity of the light to the transmitted intensity of the light so as to determine an optical density for the sample in each of the red, green, and blue channels.

46. A computer software program product according to Claim 42 further comprising an executable portion capable of directing the light source to illuminate each dye, independently of the sample, and directing a measurement of a transmitted intensity of the light transmitted therethrough in each of the red, green, and blue channels.

47. A computer software program product according to Claim 46 further comprising an executable portion capable of comparing the initial intensity of the light to the transmitted intensity of the light so as to determine an optical density for each dye in each of the red, green, and blue channels.

48. A computer software program product according to Claim 46 further comprising an executable portion capable of determining the optical density for each dye by determining a natural logarithm of a ratio of the initial intensity of the light to the transmitted intensity of the light in each of the red, green, and blue channels.

49. A computer software program product according to Claim 48 further comprising an executable portion capable of normalizing the optical density in each of the red, green, and blue channels, with respect to the channel having the highest optical

density, for each dye so as to determine the respective relative absorption coefficient in each of the red, green, and blue channels.

50. A computer software program product according to Claim 41 further comprising an executable portion capable of directing a storage media to store the relative absorption coefficient in each of the red, green, and blue channels for a plurality of dyes, and wherein the dyes indicating the molecular species in the sample are selected from the plurality of dyes.

51. A computer software program product according to Claim 50 further comprising an executable portion capable of retrieving the respective relative absorption coefficients for each of the marker dye and the counterstain from the storage media, and forming the corresponding relative absorption coefficient matrix, in accordance with the dyes indicating the molecular species in the sample which are selected from the plurality of dyes.

52. A computer software program product according to Claim 41 further comprising an executable portion capable of inverting the relative absorption coefficient matrix.

53. A computer software program product according to Claim 42 wherein the executable portion capable of directing a light source is further configured to direct the light source to provide Koehler illumination conditions.

54. A computer software program product according to Claim 41 further comprising an executable portion capable of directing the image acquisition device to correct chromatic aberration.

55. A method of determining an amount of at least one molecular specie comprising a sample, each molecular specie being indicated by a dye, from an image of

the sample captured as image data by an RGB camera in a video-microscopy system, said method comprising:

5 determining an optical density of the sample from the image data in each of a red, green, and blue channel of the RGB camera and at a pixel in the image so as to form a corresponding optical density matrix for the pixel; and multiplying the optical density matrix by an inverse of a relative absorption coefficient matrix so as to form a resultant matrix for the pixel, the relative absorption coefficient matrix comprising a relative absorption coefficient for each dye, independently of the sample, in each of the red, green, and blue channels, the resultant matrix comprising the amount of each molecular specie, as indicated by the respective dye, for the pixel.

10 56. A method according to Claim 55 further comprising determining the relative absorption coefficient for each dye, independently of the sample, in each of the red, green, and blue channels so as to form the corresponding relative absorption coefficient matrix.

15 57. A method according to Claim 56 wherein determining the relative absorption coefficient further comprises determining an initial intensity of a light emitted by a light source in each of the red, green, and blue channels of the RGB camera.

20 58. A method according to Claim 57 wherein determining the relative absorption coefficient further comprises illuminating each dye with the light source, independently of the sample, and measuring a transmitted intensity of the light transmitted therethrough in each of the red, green, and blue channels of the RGB camera.

25 59. A method according to Claim 57 wherein determining the relative absorption coefficient further comprises determining the optical density for each dye by determining a natural logarithm of a ratio of the initial intensity of the light to the transmitted intensity of the light in each of the red, green, and blue channels.

60. A method according to Claim 59 wherein determining the relative absorption coefficient further comprises normalizing the optical density in each of the red, green, and blue channels, with respect to the channel having the highest optical density, for each dye.

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61. A method according to Claim 57 wherein measuring an optical density of the sample further comprises illuminating the sample with the light source and measuring a transmitted intensity of the light transmitted therethrough in each of the red, green, and blue channels of the RGB camera.

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62. A method according to Claim 61 wherein measuring an optical density of the sample further comprises comparing the initial intensity of the light to the transmitted intensity of the light so as to determine an optical density for the sample in each of the red, green, and blue channels.

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63. A method according to Claim 61 wherein measuring an optical density of the sample further comprises calculating a natural logarithm of the ratio of the initial intensity of the light to the transmitted intensity of the light through the sample in each of the red, green, and blue channels.

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64. A video-microscopy system for determining an amount of at least one molecular specie comprising a sample, each molecular specie being indicated by a dye, from an image of the sample, said system comprising:

a microscope configured to form a magnified image of the sample;

an RGB camera having a red channel, a green channel, and a blue channel, and operably engaged with the microscope so as to be capable of forming a digital image from the magnified image;

a computer device operably engaged with the RGB camera and comprising:

a processing portion configured to determine an optical density of the sample in each of the red, green, and blue channels and at a pixel

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in the digital image so as to form a corresponding optical density matrix for the pixel; and

a processing portion configured to multiply the optical density matrix by an inverse of a relative absorption coefficient matrix so as to form a resultant matrix for the pixel of the digital image, the relative absorption coefficient matrix comprising a relative absorption coefficient for each dye, independently of the sample, in each of the red, green, and blue channels, the resultant matrix comprising the amount of each molecular specie, as indicated by the respective dye, for the pixel.

65. A system according to Claim 64 further comprising a light source directed toward the RGB camera and configured to emit a light having an initial intensity in each of the red, green, and blue channels.

66. A system according to Claim 65 wherein the light source is configured to illuminate the sample with the light and the processing portion for determining an optical density is further configured determine a transmitted intensity of the light transmitted through the sample in each of the red, green, and blue channels of the RGB camera.

67. A system according to Claim 66 wherein the processing portion for determining an optical density is further configured to determine a natural logarithm of a ratio of the initial intensity of the light to the transmitted intensity of the light so as to determine an optical density for the sample in each of the red, green, and blue channels.

68. A system according to Claim 65 wherein the computer device further comprises a processing portion configured to direct the light source to illuminate each dye with the light source, independently of the sample, and to direct a determination of a transmitted intensity of the light transmitted therethrough in each of the red, green, and blue channels of the RGB camera.

69. A system according to Claim 68 wherein the computer device further comprises a processing portion configured to compare the initial intensity of the light to the transmitted intensity of the light so as to determine an optical density for each dye in each of the red, green, and blue channels.

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70. A system according to Claim 68 wherein the computer device further comprises a processing portion configured to determine an optical density for each dye by determining a natural logarithm of a ratio of the initial intensity of the light to the transmitted intensity of the light in each of the red, green, and blue channels.

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71. A system according to Claim 70 wherein the computer device further comprises a processing portion configured to normalize the optical density in each of the red, green, and blue channels, with respect to the channel having the highest optical density, for each dye so as to determine the relative absorption coefficient in each of the red, green, and blue channels.

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72. A system according to Claim 64 wherein the computer device further comprises a storage media configured to store the relative absorption coefficient in each of the red, green, and blue channels for a plurality of dyes, and wherein the dyes indicating the molecular species in the sample are selected from the plurality of dyes.

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73. A system according to Claim 72 wherein the computer device further comprises a processing portion configured to retrieve the respective relative absorption coefficients for each dye from the storage media, and to form the corresponding relative absorption coefficient matrix, in accordance with the dyes indicating the molecular species in the sample which are selected from the plurality of dyes.

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74. A system according to Claim 64 wherein the computer device further comprises a processing portion configured to invert the relative absorption coefficient matrix.

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75. A system according to Claim 65 wherein the light source is further configured to provide Koehler illumination conditions.

76. A system according to Claim 64 wherein at least one of the microscope,
the RGB camera, and the computer device is further configured to correct chromatic
aberration.

77. A computer software program product capable of determining an amount
of at least one molecular specie comprising a sample, each molecular specie being
indicated by a dye, from a digital image of the sample captured by an RGB camera in a
video-microscopy system, said computer software program product being configured to
be executed on a computer device and comprising:

an executable portion capable of determining an optical density of the sample in
each of the red, green, and blue channels of the RGB camera and at a pixel
in the digital image so as to form a corresponding optical density matrix
for the pixel; and

an executable portion capable of multiplying the optical density matrix by an
inverse of a relative absorption coefficient matrix so as to form a resultant
matrix for the pixel of the digital image, the relative absorption coefficient
matrix comprising a relative absorption coefficient for each dye,
independently of the sample, in each of the red, green, and blue channels,
the resultant matrix comprising the amount of each molecular specie, as
indicated by the respective dye, for the pixel.

78. A computer software program product according to Claim 77 further
comprising an executable portion capable of directing a light source to illuminate the
sample with a light having an initial intensity in each of the red, green, and blue channels.

79. A computer software program product according to Claim 78 further
comprising an executable portion capable of determining a transmitted intensity of the

light transmitted through the sample in each of the red, green, and blue channels of the RGB camera.

80. A computer software program product according to Claim 79 wherein the
5 executable portion for determining an optical density is further capable of comparing the initial intensity of the light to the transmitted intensity of the light so as to determine an optical density for the sample in each of the red, green, and blue channels of the RGB camera.

10 81. A computer software program product according to Claim 79 wherein the executable portion for determining an optical density is further capable of calculating a natural logarithm of a ratio of the initial intensity of the light to the transmitted intensity of the light so as to determine an optical density for the sample in each of the red, green, and blue channels of the RGB camera.

15 82. A computer software program product according to Claim 78 further comprising an executable portion capable of directing the light source to illuminate each dye, independently of the sample, and directing a determination of a transmitted intensity of the light transmitted therethrough in each of the red, green, and blue channels of the
20 RGB camera.

83. A computer software program product according to Claim 82 further comprising an executable portion capable of comparing the initial intensity of the light to the transmitted intensity of light so as to determine an optical density for each dye in each
25 of the red, green, and blue channels.

84. A computer software program product according to Claim 82 further comprising an executable portion capable of determining an optical density for each dye by determining a natural logarithm of a ratio of the initial intensity of the light to the
30 transmitted intensity of the light in each of the red, green, and blue channels.

85. A computer software program product according to Claim 84 further comprising an executable portion capable of normalizing the optical density in each of the red, green, and blue channels, with respect to the channel having the highest optical density, for each dye so as to determine the relative absorption coefficient in each of the red, green, and blue channels.

86. A computer software program product according to Claim 77 further comprising an executable portion capable of directing a storage media to store the relative absorption coefficient in each of the red, green, and blue channels for a plurality of dyes, and wherein the dyes indicating the molecular species in the sample are selected from the plurality of dyes.

87. A computer software program product according to Claim 86 further comprising an executable portion capable of retrieving the respective relative absorption coefficients for each dye from the storage media, and forming the corresponding relative absorption coefficient matrix, in accordance with the dyes indicating the molecular species in the sample which are selected from the plurality of dyes.

88. A computer software program product according to Claim 77 further comprising an executable portion capable of inverting the relative absorption coefficient matrix.

89. A computer software program product according to Claim 78 wherein the executable portion capable of directing a light source is further configured to direct the light source to provide Koehler illumination conditions.

90. A computer software program product according to Claim 77 further comprising an executable portion capable of directing the RGB camera to correct chromatic aberration.

91. A method of correcting chromatic aberration in a video-microscopy system, comprising a light source for emitting light detectable by a magnifying objective operably engaged with an image capturing component of an image acquisition device, the video-microscopy system being configured to produce a image, said method comprising:

5 determining a centric coordinate of the magnifying objective with respect to a centric coordinate of the image capturing component;
determining, for a plurality of light wavelengths selected from the light emitted by the light source, a magnification factor for each of the plurality of wavelengths with respect to a magnification factor for a mean wavelength
10 selected from the plurality of wavelengths; and
adjusting the image such that the centric coordinate of the magnifying objective corresponds to the centric coordinate of the image capturing component and the magnification factor for each wavelength corresponds to the magnification factor for the medial wavelength.

15 92. A method according to Claim 91 further comprising disposing a slide having a calibrated grid of apertures between the light source and the magnifying objective.

20 93. A method according to Claim 92 further comprising forming an image of the grid for each of the plurality of wavelengths and with respect to a Cartesian coordinate system having an x axis and a y axis.

25 94. A method according to Claim 93 further comprising determining a centric coordinate with respect to the coordinate system for each aperture of the grid from the image for each of the of the plurality of wavelengths.

95. A method according to Claim 94 further comprising designating the image for a mean wavelength as a reference image.

96. A method according to Claim 95 further comprising determining, for each image with respect to the reference image, and for each aperture of the grid, a difference between the centric coordinate of the aperture in the respective image and the centric coordinate of the corresponding aperture in the reference image with respect to the coordinate system, the difference being expressed as a differential component along the x axis and a differential component along the y axis.

97. A method according to Claim 96 further comprising determining a linear equation minimizing a reconstruction error for each of the differential along the x axis as a function of x and the differential along the y axis as a function of y.

98. A method according to Claim 97 further comprising determining the centric coordinate of the magnifying objective by solving the linear equation for each of the differential along the x axis as a function of x and the differential along the y axis as a function of y with the differential along the x axis and the differential along the y axis being set to a value of zero for the respective linear equation.

99. A method according to Claim 98 further comprising determining a linear equation for minimizing a reconstruction error of a square root of a sum of the squares of the differential along the x axis and the differential along the y axis, as a function of the distance to the center of the magnifying objective.

100. A method according to Claim 99 wherein determining the magnification factor for the respective image with respect to the magnification factor of the reference image by determining the slope of the linear equation as a function of the distance to the center of the magnifying objective.